An Assessment of Infant Eastern Cottontail Rehabilitation Success

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ABSTRACT

Eastern cottontails (*Sylvilagus floridanus)* are the most common species brought to the Ohio Wildlife Center for rehabilitation, but many of these are likely brought in unnecessarily. Eastern cottontails raise young to bring little attention to the nest, staying away during the day, which leads people to believe infants are in need of care. In cases where this is mistaken, less adequate care is provided for the rabbits in rehabilitation facilities than they would receive in the wild from a parent. Rates of successful rehabilitation leading to release are low for this species, and infants are easily stressed and difficult to raise in captivity. Infant eastern cottontails rely on their mothers to provide gut microbes, but individuals in rehabilitation centers do not receive these microbes, leaving them more prone to infections. Goat's Milk and Goat's Milk Formula were fed in trials from March-October 2014 at the Ohio Wildlife Center Hospital, replacing the hospital's regular formulas of Pedialyte and Starter/Ultra. It was hypothesized that these formulas would better suit the needs of the rabbits and increase survival rates. All other factors of care were kept consistent, and individuals were randomly placed in trial groups for their feeding plan. The data was analyzed with logistic regression to test for the influence of feed type on survival, including a continuous covariate of weight at admission of the individuals. The formulas, however, did not significantly increase survival. Instead, weight upon admission was the only significant factor linked to successful release (0.095 ± 0.01, $p<0.01$). Because formulas cannot replace a parent's care and survival increases as eastern cottontails grow older and less reliant on formula, the main challenge rehabilitation facilities face in trying to

increase survival rates is mitigating improper human intervention in the natural history of eastern cottontails.

INTRODUCTION AND OBJECTIVES

Wildlife are forced into more interactions with humans as urban sprawl spreads. For some, increased interaction with wildlife is viewed as added aesthetic value to urban areas, but in many cases, these interactions can result in increased human-wildlife conflict (Ditchkoff 2006). Further, for the majority of wildlife, urbanization and close proximity to humans can be detrimental to their populations and decrease biodiversity (McKinney 2002). A common belief is that wildlife in urban environments are under less stress than rural populations because some effects of nature are buffered, but urban individuals face a new set of stresses (Ditchkoff 2006). Urbanization changes the landscape, consequently affecting the distribution and abundance of wildlife, as well as the resources they depend on (Blair 2004). Animals are forced to modify their behavior and life-history strategies because of human-induced stresses (Ditchkoff 2006), including reduced home ranges (Hunt 2014). Urban sprawl can also influence rates of extinction or presence of invasive species (Blair 2004). Human-induced mortalities provide additional pressures, with an estimated one million vertebrates killed each day in the United States on roads alone (Forman 1998). Roads also fragment habitats and cause behavioral changes when wildlife try to avoid road noise. Natural habitats are developed into urban environments, creating new obstacles for wildlife to avoid, such as birds flying into building windows, domestic species attacking wildlife or disturbing their nests, and harmful pesticides being sprayed on lawns (Burton 2004).

Human inhabitants of urban areas are less familiar with how to deal with wildlife species than people just 30 years ago were (Ditchkoff 2006), but with increased human-wildlife interaction, the likelihood of injuries due to these novel pressures increases, leaving the public and concerned citizens wondering how to care for these animals.

Wildlife rehabilitation, the process of caring for injured, orphaned, or sick wild animals and releasing them back to the wild when they are able to survive on their own, (Ohio Wildlife Center: What We Do 2010) provides a resource for these concerned individuals. Wildlife rehabilitation is a well-established field, with two professional organizations, the National Wildlife Rehabilitators Association and the International Wildlife Rehabilitation Council, which have set ethical guidelines, as well as guidelines for facility operation. Nevertheless, wildlife rehabilitation is a controversial practice because it can be seen as returning less "fit" individuals to a population. Because humans are now the dominant selecting force for many animal species, however, many believe there is an ethical obligation to take responsibility (DuBois 2003).

Wildlife rehabilitation is recognized as a mitigative response to wildlife morbidity and mortality, often providing care for species that have been negatively impacted by unnatural human causes (Burton 2004). A wildlife rehabilitator is responsible for and dedicated to improving the quality of care for wild animals undergoing rehabilitation (Miller 2000). Rehabilitation of wildlife incorporates prevention and control of diseases, disinfection of facilities, appropriate housing and habitat, and appropriate nutrition before pre-release conditioning, release evaluation, and finally, release (Miller 2000). Rehabilitation facilities also provide opportunities for studies of wildlife behavior, physiology, and nutrition.

One challenge faced by rehabilitation centers is lack of knowledge by the public about which animals are appropriate to bring in for care and which should be left as they are. People will observe an animal doing something they believe to be unnatural, believe it needs care, and bring it to a rehabilitation center, when the animal might actually be appropriately responding to the environment. Many infant animals thought to be orphaned are brought to rehabilitation facilities every year, though it can often be unnecessary. Lack of information to the public could be the cause for some of this.

Additionally, many see wildlife rehabilitation as an acceptable alternative to the offspring being raised by a parent of its own species. While humans can provide nourishment to the orphans, there is a lack of species recognition, sibling interaction and rivalry, and learning of wild food sources. Every attempt to return a wild animal to its proper parent should be made before hand rearing is attempted (Burton 2004).

The Ohio Wildlife Center (OWC) is a non-profit facility that provides wildlife rehabilitation to animals brought in by individuals of Ohio. The OWC has a mission of fostering awareness and appreciation of Ohio's native wildlife through rehabilitation, education, and wildlife health studies. About 5000 injured, orphaned, and ill animals representing over 100 species, are brought to the OWC's animal hospital each year for treatment (Ohio Wildlife Center: Who We Are 2010). Eastern cottontails (*Sylvilagus floridanus)*, the most abundantly admitted species, represent about 1000 of the annual admissions, or 20% (Ohio Wildlife Center: What We Do 2010).

The eastern cottontail is one of the most abundant rabbit species in North America. They breed from February to September and can have up to 5 litters per year. These litters can have 1-8 individuals, with an average size of 3-4, which are weaned at 3

to 4 weeks (Gage 2002). At birth, infant eastern cottontails weight about 25 grams, and around 115g, they begin to eat only solid food. In the wild, eastern cottontails are fed the doe's milk as infants, then eat grasses, clover, fruits, and vegetables. In the winter, the rabbit's diet consists of the woody parts of plants. In captivity, the infant rabbits are given milk-replacer formulas, then weaned to natural vegetables.

Eastern cottontails parent so as to bring little attention to their concealed nests (Burton 2004), meaning that the doe does not sit on or attend the nest as a domestic dog or cat would do with a litter (Gage 2002). The stronger scent of an adult and its larger, more visible size is more likely to attract predators (Sparks 1998), so the mother visits at dawn and dusk to feed and leaves the nest to be for the rest of the day. Well-intentioned people see infants alone in a nest and believe the nest has been abandoned, resulting in the "orphaned" animals being brought to rehabilitation centers.

Care and successful release of rabbits in rehabilitation has proven to be difficult. The OWC has a successful release rate for eastern cottontails of about 33%. Eastern cottontails do not adapt well to captivity and are extremely prone to fractures and stress (Buglass Hiss 1988). Infant rabbits, differing from other mammals, are born with a stomach and intestine that are devoid of microorganisms. Antimicrobial fatty acid is produced from an enzymatic reaction of the suckling rabbit's stomach with a substrate in the doe's milk. When the suckling rabbits are fed milk from other species or milk replacer formulas, the antimicrobial factor does not develop and the rabbits are more susceptible to infection (Buglass Hiss 1988).

It is undetermined what direct factors influence successful rehabilitation, but the correct gut physiology of infant cottontails is thought to be important for their survival.

Gut physiology can be changed by stressful situations, including, but not limited to, low feed intake, many antibiotics, unbalanced diets, feed changes, inclement weather, high humidity and cold turkey weaning (Evans 1983), leading to an overload of pathogenic bacteria (Reese "Part II" 1992). Butyric acid, caprylic acid, and capric acid are combined in a nursing cottontail's stomach to create a sterile environment, preventing growth of bacteria or fungus (Hentz 2013). In the rehabilitation process, however, these acids could become unbalanced. For example, calcium chloride is a major component in Pedialyte, which is given to the rabbits to rehydrate them. Calcium chloride can separate butyric acid from its aqueous state and devastate the rabbit's digestive system (Hentz 2013). Additionally, potassium permanganate, which can be present in tap water and on produce, oxidizes butyric and caprylic acids, rendering them useless by-products. These fatty acids are crucial in the rabbit's stomach and are important because "bunnies slowly digest the milk, which forms into a curd in their gut that can last almost 24 hours" (Hentz 2013). Keeping these acids balanced and stable is a crucial part of the health of eastern cottontails.

One suggested solution is to replace formula with raw, unpasteurized goat's milk, which is very rich in butyric, caprylic, and capric acids (Hentz 2013). A study done on a test litter found that unpasteurized goat's milk significantly increased survival of rabbits. One litter of 11 rabbits that were in good condition was split in two. One group was fed formula with bottled water. The second group was fed formula mixed with raw goat's milk. In the group fed formula with bottled water, four of the five experienced poor growth. One died by the fifth day and two were euthanized by the ninth day. In the group fed formula with goat's milk, one rabbit was lost on day five due to a feeding accident,

but all six of the rabbits experienced steady growth (Hentz 2013). This study, however, was only on one litter, a very limited sample, which is not enough to provide statistical or biological inference.

My research expanded on the study performed on the sample litter. The study addressed the influences of eastern cottontail survival in rehabilitation. Because eastern cottontails represent a large portion of admissions, staff of the OWC expressed a desire to study what could lead to higher success rates in rehabilitation. Some studies have shown the importance of diet, so the project aimed to discover if a different formula diet would play a significant role in increasing survival. In trying to identify how to better rehabilitate eastern cottontails at the OWC, the objectives of the project were: (1) To determine if a change in nutrition increased the survival rate of young eastern cottontails and (2) To determine if a change in nutrition decreased the necessary rehabilitation time of young eastern cottontails. Formula trials tested this influence. This study helps to inform rehabilitation centers, and specifically the OWC, with knowledge of influences on survival for eastern cottontails. Better understanding allows rehabilitators to focus their efforts on methods that work and increase efficiency.

METHODS

Eastern cottontail infants admitted to the OWC Hospital in Columbus, Ohio, from March-October 2014, were studied for the effects of nutrition on survival and release timing. The daily care of eastern cottontails was organized and carried out by the staff and volunteers of the OWC, and this study focused on analyzing the results of their care. Infant rabbits, those dependent on formula as a main source of food, are kept in the Nursery Ward of the hospital and fed twice a day, in the morning and evening,

mimicking natural feeding patterns of eastern cottontail mothers. The first two feedings, or initial feeding, of each individual are different from the long term feeding plan. The initial feedings are used to rehydrate the animals, while the long term plan provides nutrition. Feedings of Pedialyte, as an initial feeding, followed by Starter/Ultra, as a long term feeding plan, are what the OWC has historically fed its infant rabbits. For this study, rabbits were initially fed either Pedialyte or Goat's Milk. The long term feedings were Starter/Ultra or Goat's Milk Formula.

Once animals were admitted, they were fed and given medications as needed, until they were an appropriate weight at which to be released, 150g for eastern cottontails. During care, some individuals died or were humanely euthanized by staff. Euthanasia decisions were made if the animal was extensively suffering or developed further symptoms that would prevent rehabilitation. Only the four staff members, all of whom are licensed veterinarians or veterinary technicians, made euthanasia decisions, following established protocols of the OWC.

Outside of the formulas, all other factors of rehabilitation were kept consistent with traditional care for infant mammals. Animals were kept in cages with the litter they arrived with, or a cage with cottontails of approximately the same weight, if they were alone. Cages were placed halfway onto a heating pad so animals could be kept warm, but were able to move off the heat if they wanted. Lettuce and rabbit pellets were placed in the cage once the animals had their eyes open, which happened at about 10 days old. Animals that became ill, such as with diarrhea, were removed to be on their own so as not to allow spread to other animals. An assumption of the study is that medication use was

uniform. The study does not look at whether individuals were given medication and assumes that this was consistent across feeding groups.

Factors of feeding were kept consistent with the OWC protocol. Before every feeding, animals were stimulated to urinate. Animals were weighed to a tenth of a gram in the morning after stimulation. Rabbits were fed 10% of body weight at each of the two feedings, using the weight from the morning for both feedings. Individuals were assigned to an initial and long term feeding treatment. Eastern cottontail infants were fed with a feeding tube. A small container of formula was placed in a warm bowl of water and stirred until the formula was warm to the touch. A syringe was filled with just over 10% of the animal's body weight in warm formula. The feeding tube was attached to the syringe and formula was pushed to the end of the tube to ensure air would not be pumped into the rabbit's stomach. Outside of the rabbit, the tube was measured from the mouth to the bottom of the ribs. The tube was marked, then inserted down the throat, until the mark was at the mouth. A small amount of formula was pushed through the tube and the feeder would wait to make sure none came back out of the mouth or nose. If everything was working properly, the rest of the formula was fed to the rabbit. The tube was pinched and removed. Volunteers recorded type and amount of food given following each feeding.

Eastern cottontails above 115g do not require tube feeding and eat independently. At this weight, some went into "home care," in which a volunteer would take the animals home to feed and care for until they were ready for release at 150g.

Analyses were conducted in two stages to test for survival effects based on the initial feeding and the long-term feeding plan. Individuals were assigned to these feeding trial groups independently resulting in unbalanced samples sizes of the two conditions, so

I was not able to directly quantify the nested influences of initial and long-term feeding plans in a multi-factor framework. I used logistic regression with logit link function to test for the influence on survival of rabbits assigned to first, the initial feeding plan of Pedialyte or Goat's Milk. Further, I included a continuous covariate of initial weight of the individuals as an interaction with the formula trial to account for the potential influence of the condition of the individuals at the start of the trial. This was important to consider because an individual could be a healthy weight or extremely underweight upon admission, which likely played a role in whether they would survive, even before any formula was given. Next, I used the same basic model construct of initial weight by long term feeding group to test for differences in Goat's Milk Formula and Starter/Ultra. Significance of parameter estimates was evaluated at $\alpha = 0.05$, and parameter estimates with $0.05 < \alpha < 0.1$ were considered to be marginally significant given sample sizes.

Next, the length of stay in the rehabilitation center was also hypothesized to vary with the diet of the individuals. Because rabbits are sensitive to capture and handling, a diet that increases weight faster and reduces the time in captivity may benefit long term survival; therefore, I used a similar model structure as described above (initial weight by feeding trial) to test if the length of stay for those individuals that survived was dependent on their assigned short term and long term feeding group. In this analysis, I used a generalized linear model with Poisson distribution to model length of stay in the hospital as a response variable, where significance of parameter estimates was evaluated at $\alpha = 0.05$, and parameter estimates with $0.05 < \alpha < 0.1$ were considered to be marginally

significant given sample sizes. All data analysis was performed using Program R 2.13.x (R Development Core Team 2015).

In assessing whether formula for the initial feedings played a significant role, only those cases that were on Starter/Ultra long term were used for analysis. No cases that were on Goat's Milk Formula long term were started on Pedialyte, but cases on Starter/Ultra were started on either Pedialyte or Goat's Milk. A subset of data was created for this analysis to ensure data was not incorrectly skewed. Analysis of long term feeding plans required the same type of subset. Only cases that were started on Goat's Milk were used, as they continued to either Starter/Ultra or to Goat's Milk Formula, whereas those that started on Pedialyte were only given Starter/Ultra long term.

RESULTS

In 2014, 976 total eastern cottontails were admitted, with 863 of those either infant or juvenile in age. Feedings and dispositions of 351 eastern cottontail cases were recorded for this study. Of those, 186 achieved the requirements for analysis. Cases included for analysis in the study were less than 100g upon admission and were in the hospital for at least 2 days before disposition. This eliminated cases that had a disposition based on factors before admission rather than their formulas in care. Analyzed cases were fed only Pedialyte or Goat's Milk and Starter/Ultra or Goat's Milk Formula and had a recorded disposition of died, euthanized, or released (Table 1).

Table 1. Eastern cottontail survival at the Ohio Wildlife Center from March-October 2014, in feeding trials with initial feeds of Pedialyte or Goat's Milk and long term plans of Starter/Ultra or Goat's Milk Formula.

	Starter/Ultra	Goat's Milk Formula	Total
Pedialyte	68	1	**69**
Goat's Milk	63	54	**117**
Total	**131**	**55**	**186**

For analysis, individuals that died in care or were euthanized were grouped together. Though they are different dispositions, euthanasia is not used by the OWC unless there is a significant health problem with an animal that will likely result in death. Proportions of eastern cottontails that died and were released were consistent between all 2014 cases, the set of 2014 infants and juveniles, and the set of cases used for analysis (Figure 1).

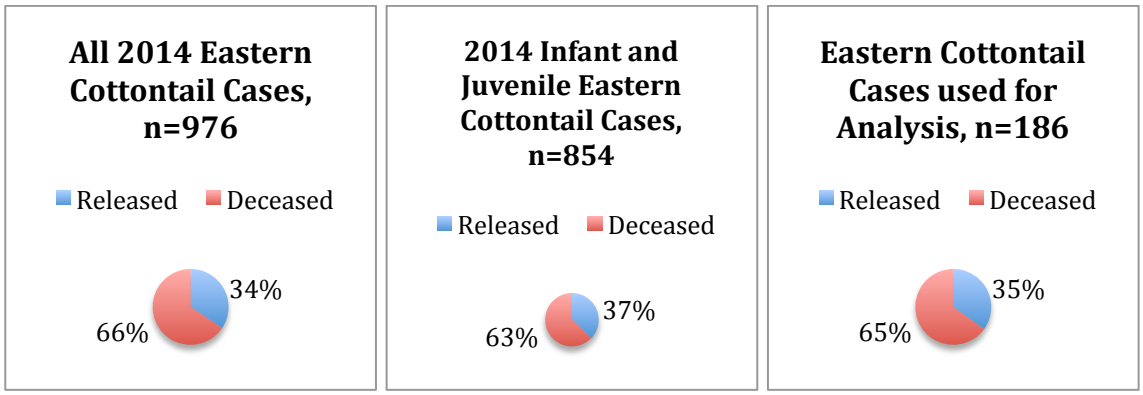

Figure 1. Eastern cottontail raw survival at the Ohio Wildlife Center from March-October 2014, showing proportions of rabbits that were released or died in care.

The first analysis was influence of initial feeding plan on survival, including initial weight as a covariate. Of the eastern cottontails fed Pedialyte, 39 died and 29 were released, while of those fed Goat's Milk, 43 died and 20 were released (Figure 2). The initial feeding of either Pedialyte or Goat's Milk did not significantly affect survival (Δfeeding trial =1.17±2.02, p= 0.562). The relationship between initial feeding trial and initial weight was also insignificant (p=0.388). Only initial weight showed a positive relationship to increased survival. (0.106±0.025, $p<0.01$).

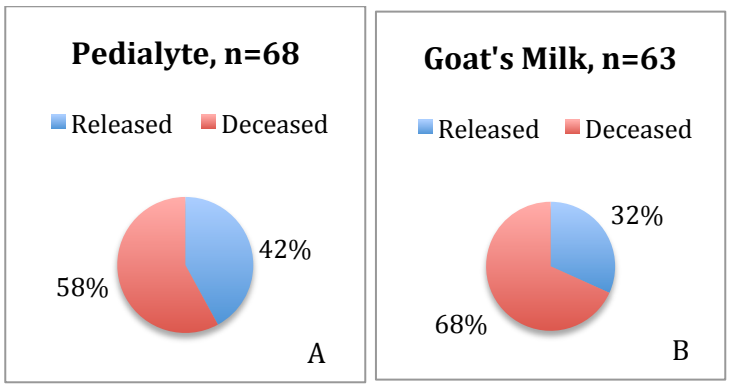

Figure 2. Eastern cottontail survival based on initial feedings of Pedialyte or Goat's Milk at the Ohio Wildlife Center from March-October 2014, showing proportions of rabbits that were released or died in care.

The next part of analysis was influence of long term feeding plan on survival, including initial weight as a covariate. Of the eastern cottontails fed Starter/Ultra long term, 43 died and 20 were released, while of those fed Goat's Milk Formula, 38 died and 16 were released (Figure 3), which was an insignificant relationship with survival (Δfeeding trial= -2.39±2.29, p=0.295). The relationship between long term feeding trial and initial weight was also insignificant (p=0.306). Only initial weight showed a positive relationship to increased survival (0.079±0.019, p<0.01).

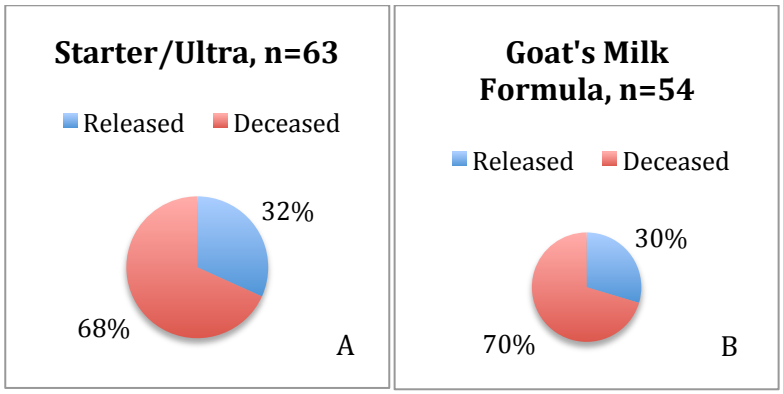

Figure 3. Eastern cottontail survival for individuals fed Starter/Ultra or Goat's Milk Formula long term at the Ohio Wildlife Center from March-October 2014, showing proportions of rabbits that were released or died in care.

Rabbits that weighed more upon entry were more likely to survive (0.095±0.01, p<0.01, Figure 4). Those fed Starter/Ultra had a slightly steeper relationship of initial

weight and probability of survival than did those that were fed Goat's Milk Formula. A change occurs around 60g with a significant increase in probability of survival.

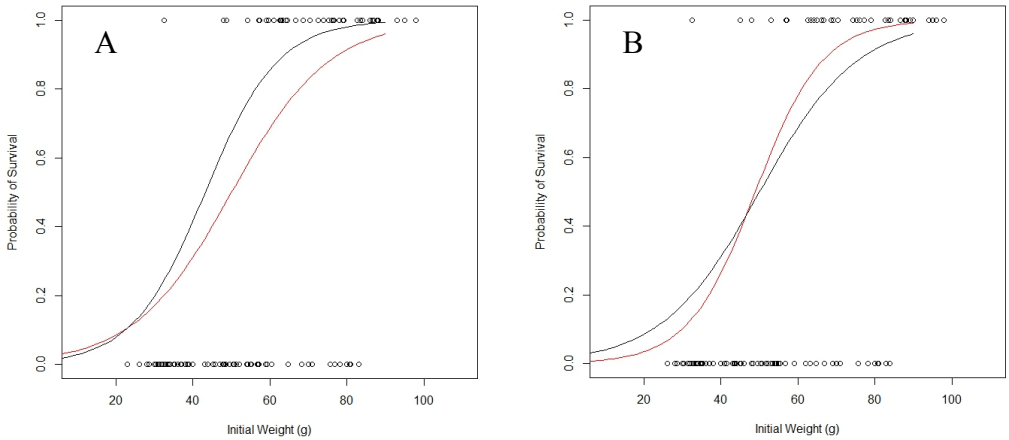

Figure 4. Eastern cottontail survival as based on weight (g) at admission at the Ohio Wildlife Center from March-October 2014. In 4A, the black line represents individuals fed Pedialyte and the red represents Goat's Milk, while in 4B, the black line represents individuals fed Starter/Ultra and the red represents Goat's Milk Formula.

Of the individuals that survived the length of the study, length of stay in hospital was not significantly different based on the initial feeding groups (-0.217±0.333, p=0.515). The same held true for the long term feedings with no significant difference in time in the hospital for the rabbits fed Starter/Ultra or Goat's Milk Formula (-0.517±0.395, p=0.191).

DISCUSSION

The results of this study indicate that the formula trials were not a significant factor in successful rehabilitation of eastern cottontails resulting in release. The different feeding trials did not offer statistical support for an increase in survival of eastern cottontails, which indicates that other factors are likely influencing low survival, but simply being in captivity could be most significantly influencing the rabbits, as eastern cottontails are difficult to care for in captivity due to their easily stressed nature (Buglass

Hiss 1988). Consistently, the main finding was that weight upon admission was the most significant variable influencing whether animals died in care or were released.

For the eastern cottontails at the OWC, changing formulas did not make a statistical difference for survival; therefore, permanent change to this formula would not be beneficial. Ease of volunteer use is another important factor in determining a method for the OWC to use in care. The Goat's Milk Formula is slightly more time consuming for volunteers to make and a little more difficult to feed to the rabbits because it is thick and difficult to push through the feeding tubes. In the midst of summer, when dozens of animals can be admitted to the hospital in a single day, utilizing volunteer time in the most efficient way possible is extremely important.

An increase in probability of survival occurs around a weight of about 60g upon admission. Many rehabilitation centers euthanize infant animals under a certain weight upon admission, allowing better care of animals that are likely to survive. The OWC could consider a protocol similar to this, setting a requirement of 50g at admission for eastern cottontails. This would decrease the workload for volunteers, allowing better care to be given to the remaining infants. This would be justified because of the very low probability of survival for those individuals admitted at under 60g.

Eastern cottontails are an r-selected species, producing many young, some of which are likely to die. In the wild, survival for eastern cottontail litters is thought to be as low as 15% (National Geographic 2015). Since the survival rate in rehabilitation centers is nearly double this, it might be as high as is possible to achieve. Rehabilitation centers should consider this as they expend time and money to care for these animals.

The importance of wildlife rehabilitation centers and their ability to successfully release animals is growing alongside human pressures on wildlife. Increasing urbanization puts humans in more frequent contact with wildlife, causing more stresses for animals in these environments (Ditchkoff 2006). Urban sprawl creates limited spaces for rabbits to nest, and increases chances of human interaction with the nest because of proximity. Wildlife change behaviors and must respond to new threats, influencing their survival (Blair 2004). Wildlife rehabilitation plays a crucial role in mitigating the impacts of wildlife-human interactions, but animals that would be better left in their natural habitat are often brought to centers for care, likely decreasing those individuals' chances at survival. Animals that might initially appear to need help might be most helped if left where they are (Sparks 1998).

Part of the problem analyzed in this study could be mediated through better contact with the public about proper protocols about when to bring animals to a wildlife hospital. Information about the natural history of eastern cottontails could be beneficial, especially characteristics of parenting that cause well-intentioned individuals to believe cottontails nests have been orphaned. At the OWC, this is accomplished through providing information on their website and the Information Hotline, a service allowing people to have their wildlife questions answered before bringing an animal to the hospital. These sources inform people how to check if an eastern cottontail nest is abandoned and what steps to take if it is. This information should be made available to anyone who brings eastern cottontails to the hospital, so they are informed for future situations, or so a reunion with the mother can be attempted, if possible.

Some bias was present in the trials for this study. Animals were often, though not always, fed the same diet as the animals in the cage with them. In instances where littermates were in a cage together and were fed the same formula, results could have been biased due to relatedness. In future studies, this could be addressed by splitting litters in half and assigning them to different feeding trials. Distributing initial weights evenly across feeding groups could further enhance this.

Additional bias could have come from many volunteers feeding each animal. This was likely mostly even across groups; however, this could have had a greater influence on the Goat's Milk Formula group because it was a more difficult formula to use, potentially leading to more feeding errors by volunteers. If possible, a single volunteer would carry out all of the feedings, so they are done consistently, rather than by different volunteers at each feeding, but this would not eliminate the bias of the varying challenges of using the formulas.

A limitation of the study is that some cases died directly due to being in care, such as mistubing, resulting in a negative disposition not related to nutritional or age factors. 36% of eastern cottontails that died were euthanized in care. As discussed earlier, this was done following the OWC protocols and only when the animal was likely to die. Another limitation is that this study only analyzed successful release from captivity, but did not evaluate success after release. A future study with post-release monitoring would evaluate impacts of the population.

Control of the type and amount of solid food after being weaned off of formula would allow more certainty regarding influences of food after formula. Additional types of formulas targeting various nutritional needs of the eastern cottontail and more evenly

distributed sample groups would allow additional analysis of the effects of formulas. It would be useful to sample gastrointestinal microbes before, throughout, and after formula trials to see the influence of formula on these gut communities. This would inform on whether the formula was meeting the normal flora needs, but something else was unmet, or if the rabbits were still in need of microorganisms. Another factor that could be tested is stress levels of eastern cottontails in rehabilitation compared to those in natural settings. Each additional study will provide valuable information about eastern cottontails and increase knowledge of how to care for them.

Rehabilitation centers play a crucial role in mitigating the impacts humans have on wildlife. The volunteers of the OWC rehabilitate and raise Ohio native species to the best of their ability, but in the case of infants, human care cannot replace that given by its own species. These centers must manage volunteers to organize efforts and protect wildlife, but ultimately, wildlife rehabilitation centers must manage the general population of caring citizens so that animals can be in the situation that is best for them.

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